Super-resolution microscopy by sub- and super-Poissonian photon statistics

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Improving spatial resolution is critical in current biological and medical research [1]. Within the principle of optical diffraction, Ernst Abbe initially identified the optical resolution limits as imposed by the numerical aperture of the objective and the wavelength of light [2]. In the early 1990s, novel approaches broke beyond Abbe's limit by circumventing some of its underlying assumptions (explicit or implicit), among them the linear and static response of the sample to incident light, the far-field intensity detection and uniform illumination. By exploiting the non-linear or randomly selective response of point-like fluorescence markers, pioneering techniques such as stimulated emission depletion (STED) microscopy and single-molecule localization microscopy (SMLM) have substantially advanced the field and demonstrated exceptional resolution enhancement [3,4].

However, when using photosensitive samples these methods can be of limited use. Biologists often demand live cell imaging technologies that deliver high resolution while minimizing photodamage, which posed some questions about the reliability of these super-resolution methods for bio-applications [5].

A bio-compatible super-resolution approach, that does not require high illumination levels, is the super-resolution optical fluctuation microscopy (SOFI) [6,7], which exploits the natural or induced random fluctuation of fluorophores' brightness. SOFI is based on a semi-classical model of light that does not take into account quantum fluctuation, and leads to a strongly limited resolution enhancement in low light scenarios. In low light regime only a full quantum description provides the route to reach the optimal performance in optical imaging, often leveraging on peculiar quantum features. The genuine quantum anti-bunching properties of single photon emitters have been used to achieve super-resolution by measuring higher order correlation function of the photons [8,9]. These last methods can be seen as the equivalent of SOFI but for sub-Poissonian emitters.

This talk aims to introduce a full quantum method for super-resolution named quantum super-resolution imaging by photon statistics (QSIPS) [10] that is based on a rigorous full quantum model describing the photon emission and detection. Our model yields to a generalized SOFI approach that works optimally at any intensity level and with any non-Poissonian emitter, including single photon emitters and any kind of blinking (photoswitching) fluorophores. Here we present the model together with simulations, and experimental results, demonstrating the superiority of our approach over traditional SOFI methodologies.

Furthermore, we present the integration of the QSIPS method, with Structured Illumination Microscopy (SIM) [11]. In SIM the sample is illuminated with a set of non-uniform patterns, and the final super-resolved image is reconstructed by the combination of all the single pattern images. The integration of non-Poissonian superresolution methods with SIM has been demonstrated theoretically to provide a super resolution scaling J+J1/2 being J the correlation order [12]. Here, we demonstrate that even in combination with structured light our QSIPS method outperforms the classical implementation with SOFI, especially in scenarios characterized by low light illumination

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